CHEMICAL CHARACTERISTICS OF DISSOLVED ORGANIC MATTER IN RIVER WATER

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CHEMICAL CHARACTERISTICS OF DISSOLVED

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SUMMARY

Dissolved organic matter from the Satilla River, a Coastal Plain river of Southeast Georgia, and a tributary swamp was separated into chromatographically homogeneous fractions by 'Sephadex' gels. The organic matter and its fractions were characterized by the following parameters: (1) elemental composition, (2) oxygen-containing functional group content, (3) infrared spectra, and (4) number-average molecular weight ($\overline{Mn}$).

In general, the oxygen-containing functional group content of each fraction increases with decreasing $\overline{Mn}$. Total acidity ranges from 7.3 to 12.9 meq/g, and carboxyl group content from 3.2 to 9.5 meq/g. River water organic matter close to a swamp source shows lower total acidity and carboxyl group content when compared to organic matter further downstream. Carbon ranges from 40.0 to 54.3 percent, hydrogen from 3.4 to 5.6 percent, nitrogen from 0.7 to 1.9 percent, and oxygen from 39.6 to 55.3 percent. Infrared spectra of the fractions show the 1725 cm^{-1} band becoming progressively stronger with increasing carboxyl concentration. The weak 1725 cm^{-1} band in the spectra of undesalted organic matter indicates that the carboxyl groups are the most reactive of the functional groups in organic matter-salt formation.

The $\overline{Mn}$ of fractions as determined by vapor pressure osmometry ranged from 528 to 3095. Based on the $\overline{Mn}$ of each fraction, the $\overline{Mn}$ of the unfractionated organic matter calculates to be 1322, and is in close agreement with the $\overline{Mn}$

of 1266 determined on the original material. The ratio $\overline{Mw}:\overline{Mn}$ ($\overline{Mw}$ = weight-average molecular weight) calculated from the fractions is close to unity, indicating that the parent material is of low polydispersity.

The fraction with the highest $\overline{Mn}$ is similar to humic acid, but accounts for only a small portion (~5%) of the total organic matter, the rest of which resembles fulvic acid.

Humic substances of a rather narrow molecular weight range are leached from a swampy, low relief area. As they are transported downstream, it is expected that the higher $\overline{Mn}$ portion is removed from solution by flocculation. The soluble organic matter remaining will exert control on the water chemistry of the river through exchange reactions with the inorganic constituents and through dissociation. Since the transportation and accumulation of toxic pollutants such as heavy metals is greatly influenced by their association with river water organic matter, the results of this study should be of interest to those concerned with environmental problems.

CHAPTER I

INTRODUCTION

Most of the dissolved organic matter (DOM) in Coastal Plain rivers of Georgia is derived from water percolating through soil or water discharged from swamps by flushing. In a recent study of some Coastal Plain rivers of the Southeastern United States (Beck et al., 1973), chemical characteristics of the dissolved organic matter showed a marked similarity to those of humic substances. Humic substances occur in soils, terrestrial and marine sediments, and natural waters. Chemically and physically, humic substances differ greatly from the homogeneous classes of organic compounds found in living matter. Classification of humic substances has been based on solubility, the two main classes being humic acid and fulvic acid. Both are soluble in weakly alkaline solution. Upon acidification, humic acid precipitates, and fulvic acid remains in solution.

The formation process of humic substances is complex and not well understood. Described by Felbeck (1971), the synthesis of humic substances involves monomers derived from the decomposition of plant material and metabolic products of microbial origin, and subsequent polymerization of monomers into high molecular weight material containing polyphenolic and polycarboxylic building blocks (Schnitzer and Khan, 1972). Most investigators characterize humic substances according to elemental composition, content of oxygen-containing

functional groups, ultraviolet, visible, and infrared spectra, and molecular weight. In general, when compared to humic acid, fulvic acid has higher total acidity, higher carboxyl group content, and lower phenolic hydroxyl content. Fulvic acid has lower carbon content, higher oxygen content, and lower average molecular weight than humic acid.

The bulk of dissolved organic matter in natural water consists of humic substances leached from the soil. Packham (1964) found that most of the organic matter in the Thames River was fulvic acid. According to Midwood and Felbeck (1968), fulvic acid was the major constituent of pond water. Based on degradative studies, Christman and Ghassemi (1966) concluded that dissolved organic matter from natural waters partly consists of polycarboxylic acid with a phenolic nucleus. Oxidative degradation products of dissolved organic matter are very similar to the degradation products of wood and soil organic matter.

River water organic matter has the ability to interact with inorganic dissolved species by adsorption, cation exchange, and chelation, and to donate hydrogen ions in the process, thus influencing the chemistry of natural waters (Beck et al., 1973). In this study dissolved organic matter in river water was characterized by chemical and spectroscopic analyses. Only a limited attempt was made to separate the originally polydisperse (heterogeneous) organic matter into more homogeneous fractions for detailed study. The purpose of this study is to continue the characterization of river water organic matter previously conducted by Beck et al. (1973) in order to better understand the influence of dissolved organic matter on the chemistry of river water. Particular emphasis

is placed on the preparation and analysis of chromatographically homogeneous fractions, and the determination of the average molecular weights of the original material and its fractions.

CHAPTER II

MATERIALS AND METHODS

Sample Collection and Extraction

The Satilla River lying in the Coastal Flatwoods region of the Coastal Plain receives its water from the internal discharge and surface runoff of waters from low lying soils and swamps. The geologic setting of the Satilla River Basin has been described in detail by Bergeaux (1969). Samples were collected from two locations, RS19 and RS36 (Figure 1). RS19 is situated in the Satilla River just downstream from where its many branches join. RS36 is located downstream from RS19 in the County Line Branch, a small stream flowing through a swamp into the Satilla River (between Camden and Brantley Counties, Georgia).

A sample (RS19) of 120 liters of Satilla River water was collected July, 1972 in twenty-liter polyethylene containers. The river water was centrifuged at 12,000 r.p.m. on a Sorvall centrifuge (Model SS-3, rotor type SS-34) equipped with a continuous flow system (KSB-3, flow rate: 100-150 ml/min.) to remove particulate matter. The river water was concentrated five-fold in vacuo and stored at 3^{o}C under nitrogen. The concentrate was desalted with 'Rexyn 101' (H^{+} form). Analysis by atomic adsorption spectrometry before and after desalting showed that most of the sodium ion was removed. The volume of the desalted concentrate was reduced further to one liter and freeze-dried.

River water (240 l) from location RS36 was collected in August, 1972.

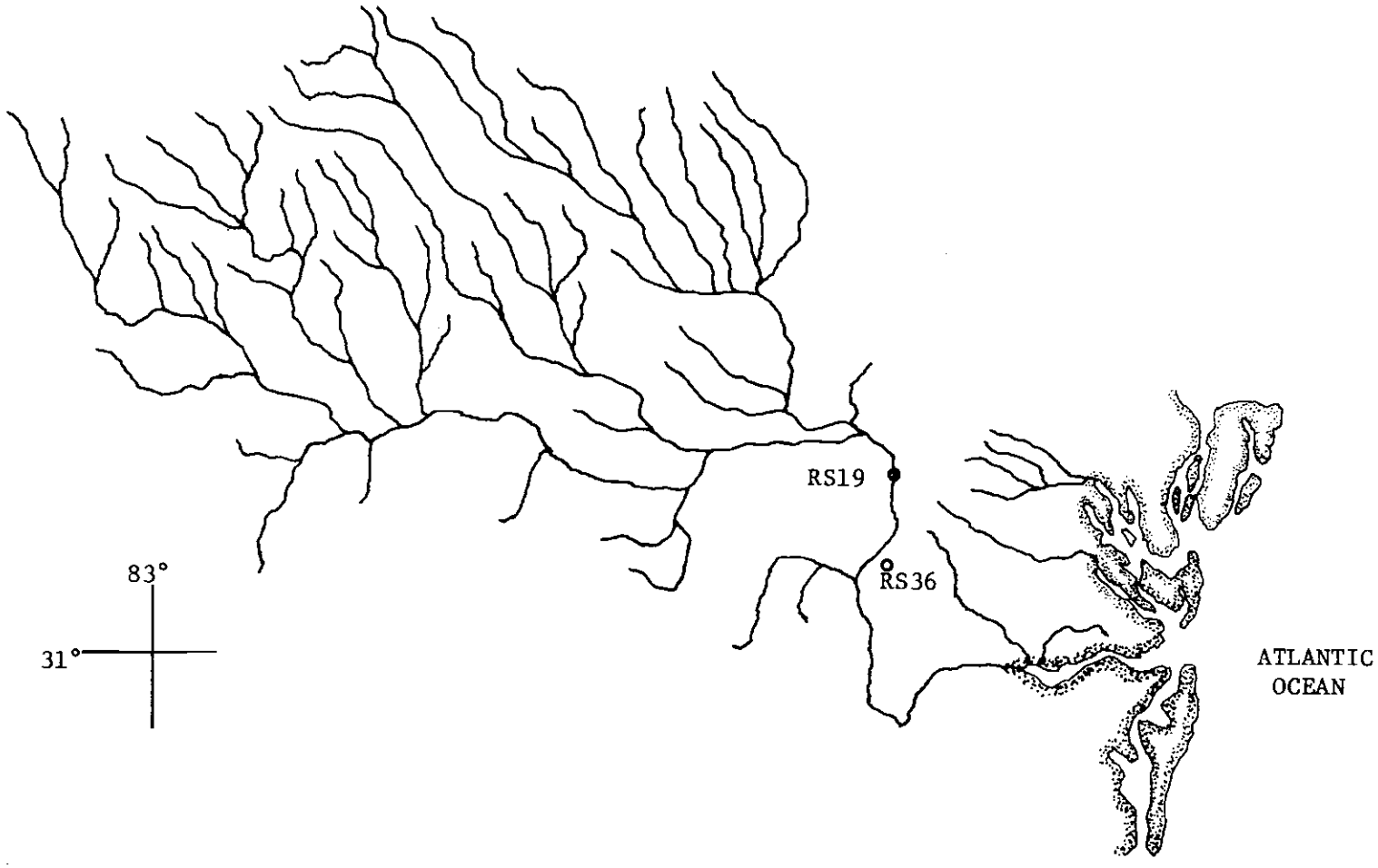

Figure 1. Sample Location, the Satilla River Basin.

The river water was concentrated in vacuo and freeze-dried yielding the dissolved chemical load minus volatile acids. The dry sample was called RS36A RAW. Distilled water was added to RS36A RAW (50 ml H_2O/gram organic matter) yielding two fractions: a) organic matter dissolved in water, and b) a dark precipitate called RS36A RAW PPT. The precipitate was removed by centrifugation at 12,000 r.p.m. for 15 minutes. The dissolved fraction of RS36A RAW was desalted twice by the batch method with 'Bio-Rad AGX8 50-100'(H^+ form) using a wet bed volume of 25 ml resin to 1 gram organic matter. The final solution was concentrated three-fold in vacuo and freeze-dried,yielding dry, desalted organic matter called RS36A. Before freeze-drying, the dissolved fraction contained HCl and H_2SO_4 from the river water anions (Cl^-, and SO_4^-). These should be removed by freeze-drying and subsequent drying in vacuo.

A second sample (240 l) collected in late August, 1972 from location RS36 was concentrated and freeze-dried. This material (undesalted) was called RS36B RAW.

Gel Filtration Chromatography (GFC)

The chromatographic assembly is shown in Figure 2. Columns were prepared with 'Sephadex' gels, G-50, G-25, G-15, and G-10. Degassed distilled water was pumped from a reservoir by a controlled volume pump (Milton Roy Mini-Pump Model 196-32) to either a column of dimensions 2.5 x 100 cm or 4.5 x 50 cm. To reduce cyclic pressure gradients caused by the reciprocating pump, a pulse dampener (LDC Model 709) was connected via a flow restrictor creating a back pressure of 300-350 p.s.i. The flow rate was 40 ml/hr. for the small

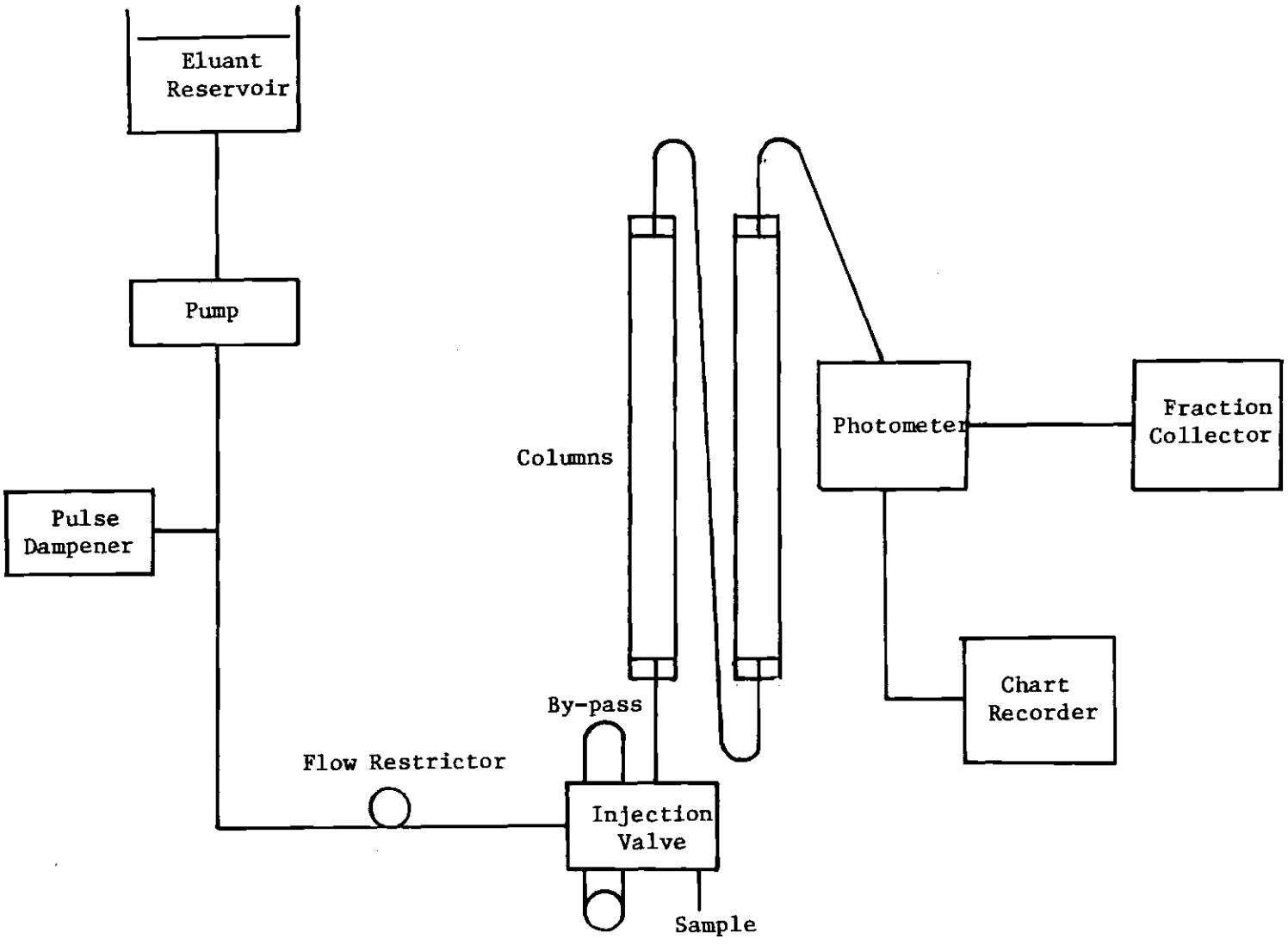

Figure 2. Chromatographic Assembly for the Gel Filtration of DOM.

diameter column, and 152 ml/hr. or 120 ml/hr. for the large diameter column. The volume of each sample injected onto the column was standardized by means of a sample loop of a volume of 10 ml for the smaller column, and 40 ml for the larger column. The optical density of the column effluent was continually monitored by a flow-through photometer (ISCO Model 222) at 425 mμ and recorded on a strip chart recorder. Five ml fractions and 15 ml fractions from the smaller and larger columns respectively, were collected in test tubes on a fraction collector (ISCO Model 326).

To prepare RS19 for GFC, one gram of freeze-dried, purified RS19 was dissolved in 150 ml of distilled water. The organic matter went into solution readily. After adjusting to pH 7.0 with 0.1N NaOH, distilled water was added to a total of 200 ml. Ten ml portions were injected onto the smaller column. The GFC fractions obtained by the smaller column did not yield sufficient material to carry out molecular weight determinations. Consequently, the larger column was used. GFC of RS36A was performed in a manner similar to the GFC of RS 19. A total of 6.290 g of organic matter was fractionated on the larger column in portions of 40 ml containing 0.24 g of organic matter.

After chromatography on one gel, the effluent was separated into two fractions, the excluded material and the retarded material (Figure 3). In some cases the excluded material had to be rechromatographed, because it contained retarded material yielding fractions Excluded II and Retarded II. The excluded material supposedly passes around the individual gel particles and is the first to leave the column. The retarded material passes through the porous gel particles, is retarded, and is eluted from the column after the excluded material. The

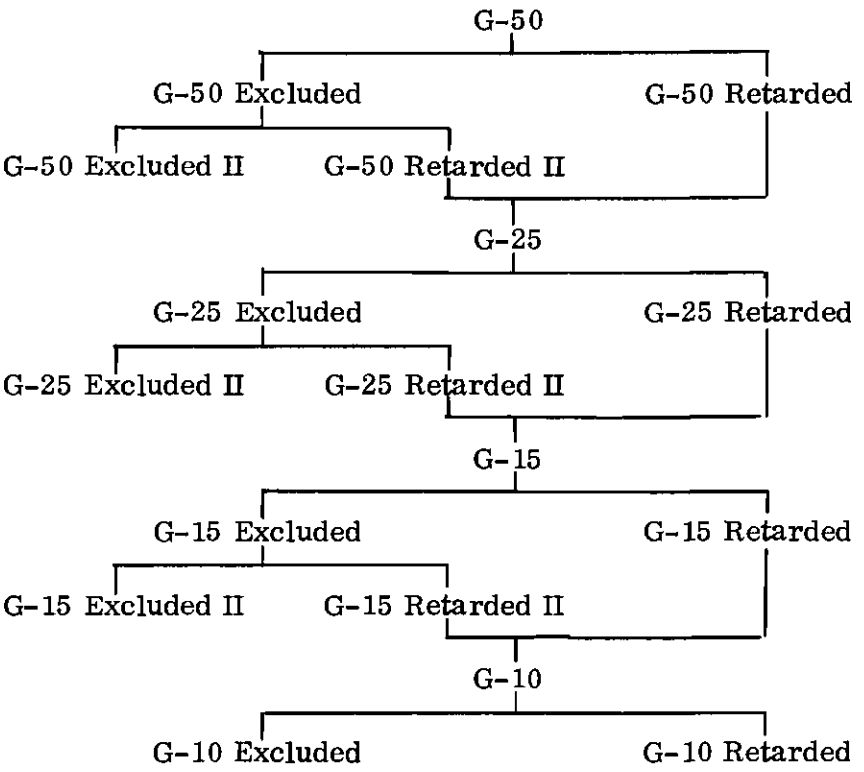

Figure 3. Schematic Flow Chart for Gel Filtration of DOM.

order of gels used was according to their exclusion limit for a particular range of average molecular weights, the gel with the highest exclusion limit (G-50) being used first and the gel with the lowest exclusion limit (G-10) being used last. The excluded material from G-50 and G-15 gels contained retarded material and was chromatographed again to obtain a more homogeneous separation. The excluded material from G-25 and G-15 did not yield retarded material upon rechromatography.

Partition coefficients (K_D) used to describe the elution behavior of organic matter on 'Sephadex' gels were calculated according to Gelotte (1960) as follows:

$$K_D = \frac{V_e - V_o}{V_i} \text{ , where } V_i = a \cdot W_r \text{ .}$$

V_i is the aqueous volume inside the gel grains, V_o is the outer aqueous volume surrounding the gel grains, and V_e is the elution volume. V_i was calculated by multiplying the amount of water regained (W_r) by the weight of the dry gel (a). V_o was determined with 'Dextran Blue 2000' using 20 mg Dextran Blue/10 ml phosphate buffer (50 ml of 0.1 molar potassium dihydrogen phosphate + 29.1 ml 0.1 molar NaOH) and eluting with phosphate buffer at pH 7.0. Partition coefficients of elution peaks near 0.0 represent excluded material. Partition coefficients greater than 0.0 represent retarded material.

Elemental Analysis

Carbon, hydrogen, and nitrogen analyses of dissolved organic matter and GFC fractions were performed by a commercial analytical laboratory (Atlantic Microlab, Inc., Atlanta, Georgia). For the determination of carbon and hydrogen,

a modified Pregel technique was used. Nitrogen was analyzed by a modified Dumas technique. Samples were dried to constant weight in vacuo over P_2O_5 for several days before analysis.

Oxygen-Containing Functional Group Analysis

Analyses of total acidity and carboxyl group content were performed according to Schnitzer and Gupta (1965). Total acidity was determined by the barium hydroxide method. The calcium acetate method was used to measure the carboxyl group content.

In the barium hydroxide method an excess of 0.25 N $Ba(OH)_2$ is reacted with dissolved organic matter. The remaining $Ba(OH)_2$ is titrated with 0.10N HCl to pH 8.4 using a glass electrode. A blank is prepared and titrated in a similar manner. Total acidity is calculated as follows:

$$\frac{\left(\text{titer}_{(\text{blank})} - \text{titer}_{(\text{sample})}\right) \times \text{grams HCl} \times \text{density HCl} \times \text{normality} \times 1000}{\text{weight of sample (mg)}}$$

= milliequivalents (meq) total acidity/gram organic matter.

For greater precision, titrations were performed on a Mettler balance P160-titration system DP11.

For the determination of carboxyl groups, 1.0 N calcium acetate is reacted with dissolved organic matter. The reaction yields acetic acid which is titrated with 0.010 N NaOH to pH 9.8 using a glass electrode. A blank is prepared and titrated. Carboxyl group content is calculated as follows:

$$\frac{\left(\text{titer}_{(\text{sample})} - \text{titer}_{(\text{blank})}\right) \times \text{grams NaOH} \times \text{density NaOH} \times \text{normality} \times 1000}{\text{weight of sample (mg)}}$$

= meq carboxyl groups/gram dissolved organic matter.

Throughout the preparation and subsequent handling of NaOH and particularly $Ba(OH)_2$, extreme care was exercised to prevent absorption of atmospheric CO_2. Only carbonate-free distilled water was used. Titrations were performed under nitrogen.

Infrared Spectroscopy

Infrared spectra for RS36A RAW, RS36B RAW, RS19, and RS36A were recorded on a Beckman IR-12 spectrophotometer. The other samples (RS36A RAW PPT, RS19 fractions and RS36A fractions) were analyzed on a Perkin-Elmer 457 spectrophotometer. The organic matter was dried over P_2O_5 *in vacuo* for several days to constant weight. Spectroanalytical KBr was air dried for 3 hours at 105-110°C. KBr pellets were prepared under the precautions outlined by Theng *et al*. (1966). Approximately 1-2 mg organic matter was mixed with 100-150 mg KBr, the mixture ground to a fine powder and pressed under 10,000 p.s.i. for several minutes. After several runs, a KBr blank spectrum was recorded. In all cases, moisture interference from KBr was found to be minimal.

Vapor Pressure Osmometry

The method used for the determination of number-average molecular weights was developed by Hansen and Schnitzer (1969). Number-average molecular weight ($\overline{Mn}$) is the ratio of the total weight of molecules present in a mixture to the total

number of molecules. $\overline{Mn}$'s were determined for the original organic matter (RS36A Parent)[1] and each fraction of RS36A on a Mechrolab Model 301A vapor pressure osmometer at 37°C. The instrument was calibrated according to the manufacturer's recommendations. The calibration constant was determined to be 60.31 kg ohms/g. For each of samples RS36A Parent, G-25 Excluded, and G-10 Retarded, solutions were prepared with 10g H_2O. Samples RS36A G-50 Excluded, G-15 Excluded, and G-10 Excluded were prepared with 5g H_2O. Weights of organic matter and distilled water were measured to 0.1 mg on a Mettler balance (Type H-15). ΔR values (instrument readout in ohms) were recorded 10 minutes ($\pm$ 10 sec.) after placing a drop of solution on the thermistor bead. Three to six readings were taken for each concentration. Distilled water from the same container was used in the solvent syringe, solvent cup, and for the preparation of the solutions. After cleaning, each syringe was rinsed once with sample solution before loading for injection. After two to three readings, the osmometer was re-balanced with solvent.

pH was measured with a combination glass electrode (Sargent Model S-30072-15) on an Orion 801 Digital pH meter. Two standard buffer solutions, 0.05 molal potassium hydrogen phthalate (pH 4.008 at 25°C, Fisher Scientific Co.) and 0.05 molal potassium tetroxalate (pH 1.679 at 25°C, National Bureau of Standards) were used to calibrate the pH electrode. All readings were recorded three minutes after insertion of the pH electrode into the solution. Readings of

[1] For clarity, samples RS19 and RS36 are sometimes referred to as RS19 Parent and RS36A Parent to distinguish them from their GFC fractions.

pH of each solution used in the vapor pressure osmometer were recorded in the following sequence: 1) pH of standard buffers, 2) pH of various concentrations of one fraction, 3) pH of standard buffers, 4) pH of various concentrations of one fraction, and 5) pH of standard buffers. The average value for the pH of the standard buffers was determined and corrected by comparing the recorded value of the standard buffers with their standard pH, i.e. 1.679 and 4.008 at 25^{o}C. A correction was then applied to the average pH reading of each concentration. The corrected pH value was calculated to the nearest 0.001 unit.

CHAPTER III

RESULTS AND DISCUSSION

Dissolved Organic Matter in River Water

Desalting and freeze-drying of 120 liters of river water yielded 4.93 g of fluffy, dark brown material (RS19) which is a concentration of 34.7 ppm on an ash-free basis. Beck et al. (1973) found 24.0 ppm organic carbon in a water sample from the same site. Based on the approximation that the organic matter is 50 percent carbon, the concentration of organic matter is 48.0 ppm. The low yield (34.7 ppm) is caused partly by adsorption of organic matter on the ion exchange resin, and partly by the many different manipulations.

River water from the County Line Branch of the Satilla River yielded 31.2 g (RS36A) after concentration and freeze-drying. The concentration of RS36A on an ash-free basis was 120 ppm. When the sample was redissolved in distilled water, a dark precipitate (RS36A RAW PPT) formed. RS36A RAW PPT weighed 1.483 g and accounted for 3.0 percent of the total organic matter (ash-free). In order to check whether humic acid had been adsorbed on the ion exchange resin during desalting of RS36A, the resin was leached with 2N NaOH. The leachate was first acidified to pH 3, then to pH 1 with concentrated HCl. No precipitate formed and the solution remained a brown color. The absence of precipitation indicates that humic acid was not adsorbed on the resin. Probably only material resembling fulvic acid was adsorbed. Another water sample from

the County Line Branch (RS36B) yielded 37.5 g organic matter. Its concentration in river water was 132 ppm (ash-free).

Gel Filtration Chromatography

Gel filtration chromatography (GFC) has been applied by numerous investigators to separate humic substances into fractions (Gjessing and Lee, 1967; Ghassemi and Christman, 1968; Schnitzer and Skinner, 1968; Swift and Posner, 1971). 'Sephadex' gels are used to separate dissolved organic matter into fractions of a particular molecular weight range. Since interactions between the gel and organic matter occur (Cameron, et al., 1972), only those fractions which have the least chance of interaction, i.e. the excluded material,were considered to be chromatographically homogeneous.

Figure 4 shows the elution patterns of various GFC fractions. The dashed lines in Figure 4 indicates the separation made between fractions. The material to the left of the dashed line is of low K_D value and was considered to be excluded. The material to the right was considered retarded. Partition coefficients (K_D) for the excluded material of RS36A were not determined with 'Dextran Blue 2000' as had been done previously for RS19. A K_D value of 0.0 was assigned to the excluded peaks of the RS36A fractions. For the elution peaks in Figure 4, K_D values are less than 1.0 indicating that adsorption between gel and organic matter was minimal (Schnitzer and Skinner, 1968).

In order to investigate interactions between dissolved organic matter (DOM) and gel, the excluded material from RS19 G-25 was chromatographed as shown by the elution pattern of G-25 Excluded (see Figure 4). The excluded

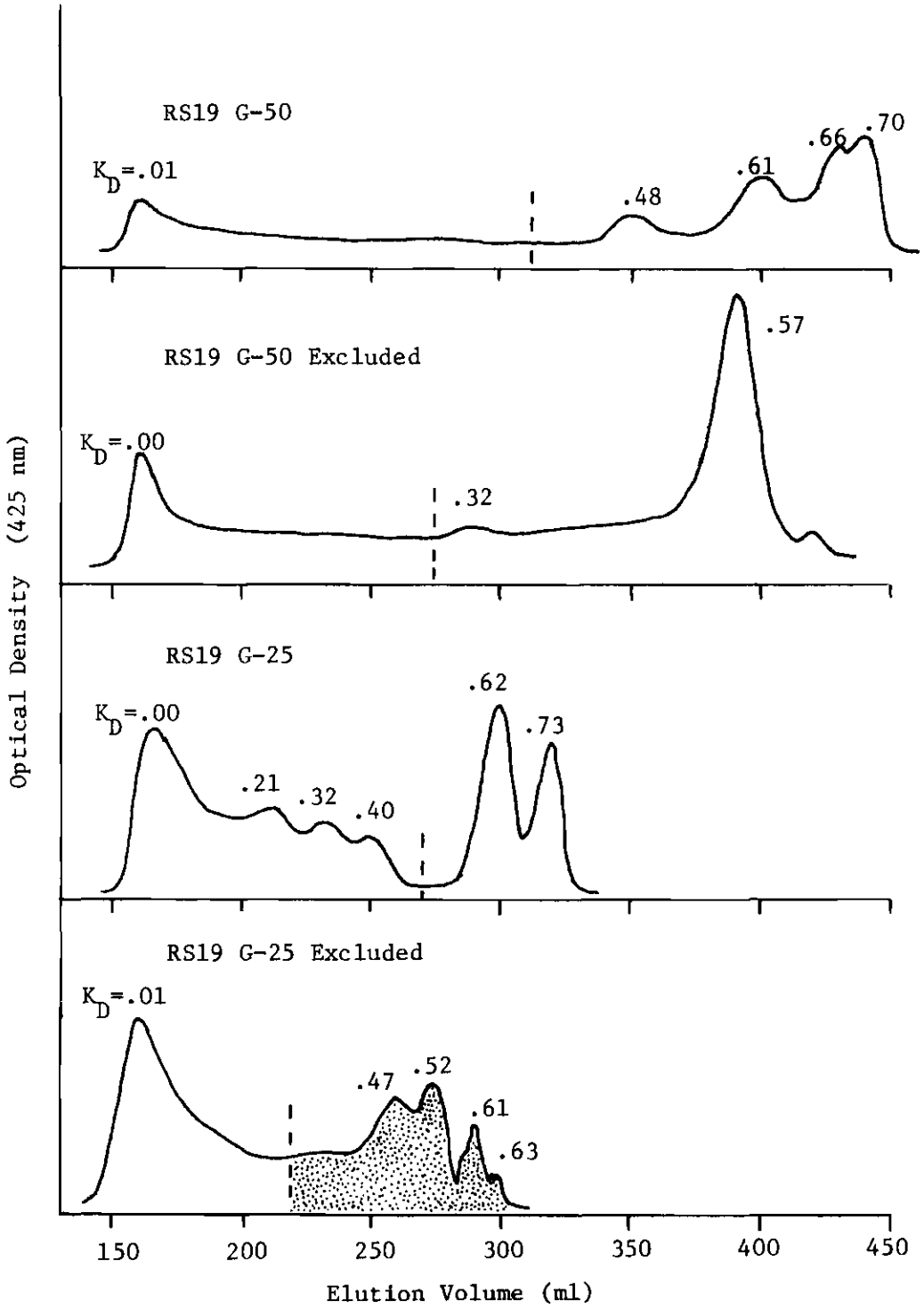

Figure 4. Elution Patterns of DOM. RS19 Fractions Eluted on 2.5 x 100 cm RS36A Fractions Eluted on 4.5 x 50 cm Column.

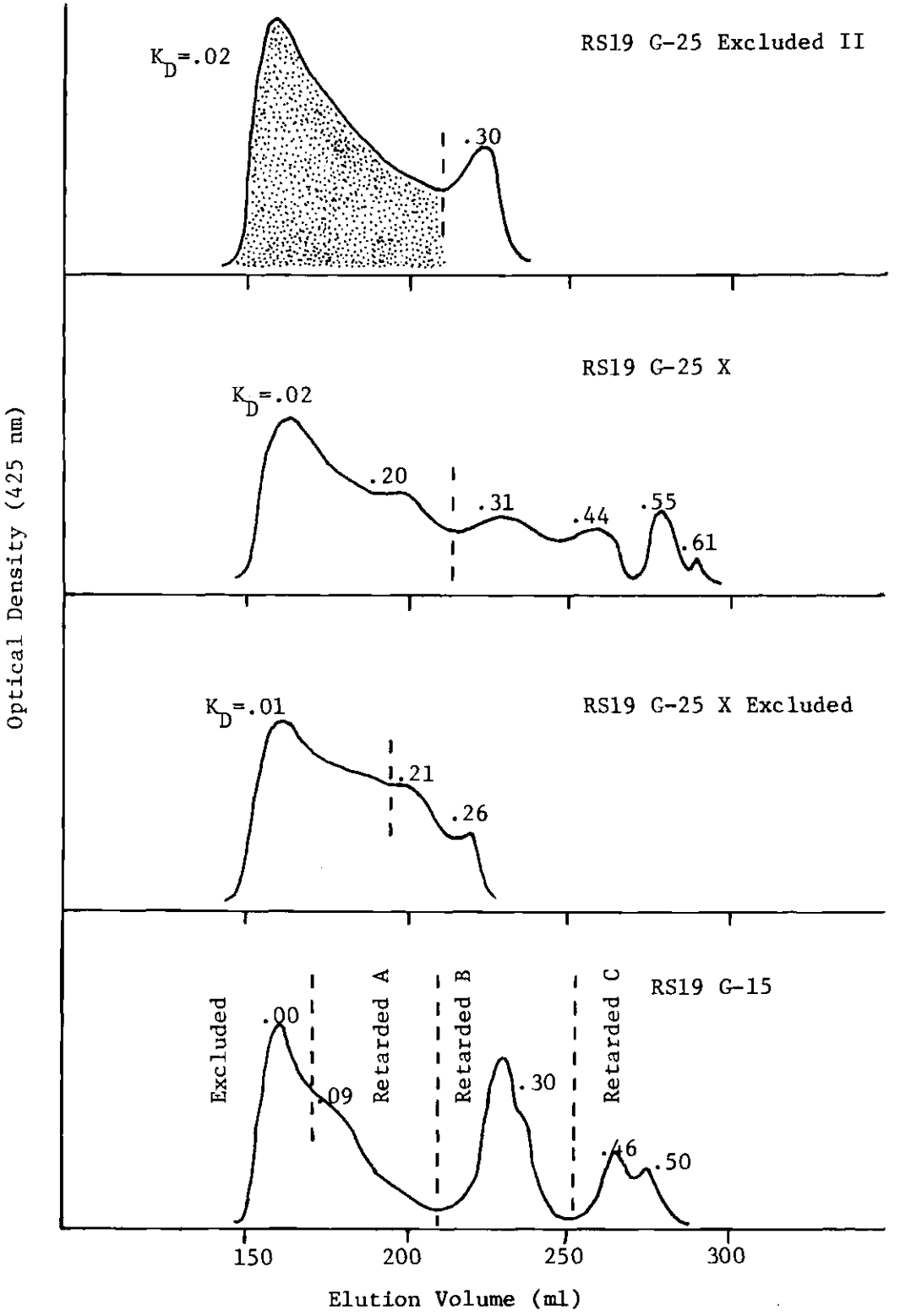

Figure 4 (Continued). Elution Patterns of DOM. RS19 Fractions Eluted on 2.5 x 100 cm RS36A Fractions Eluted on 4.5 x 50 cm Column.

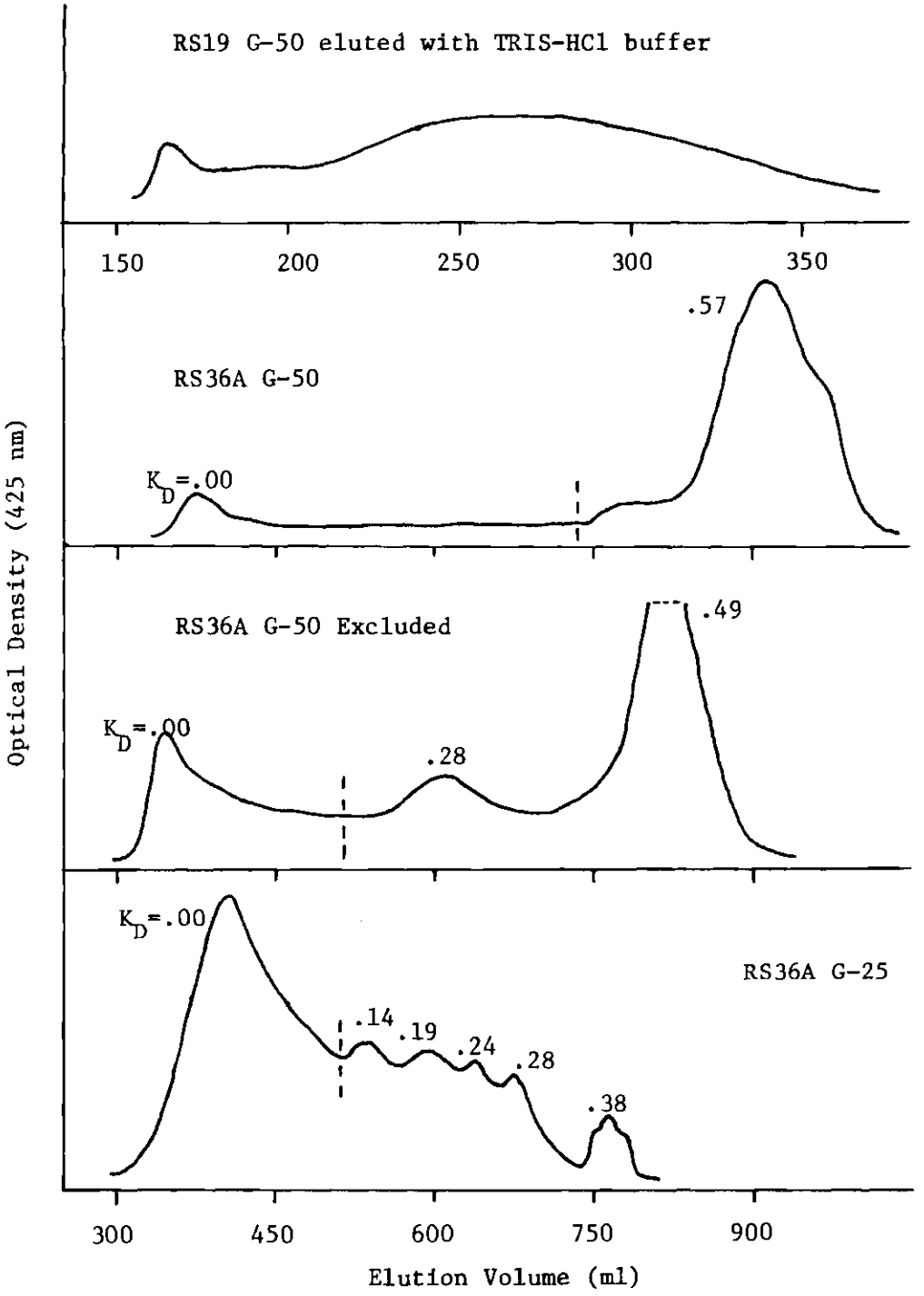

Figure 4 (Continued). Elution Patterns of DOM. RS19 Fractions Eluted on 2.5 x 100 cm RS36A Fractions Eluted on 4.5 x 50 cm Column.

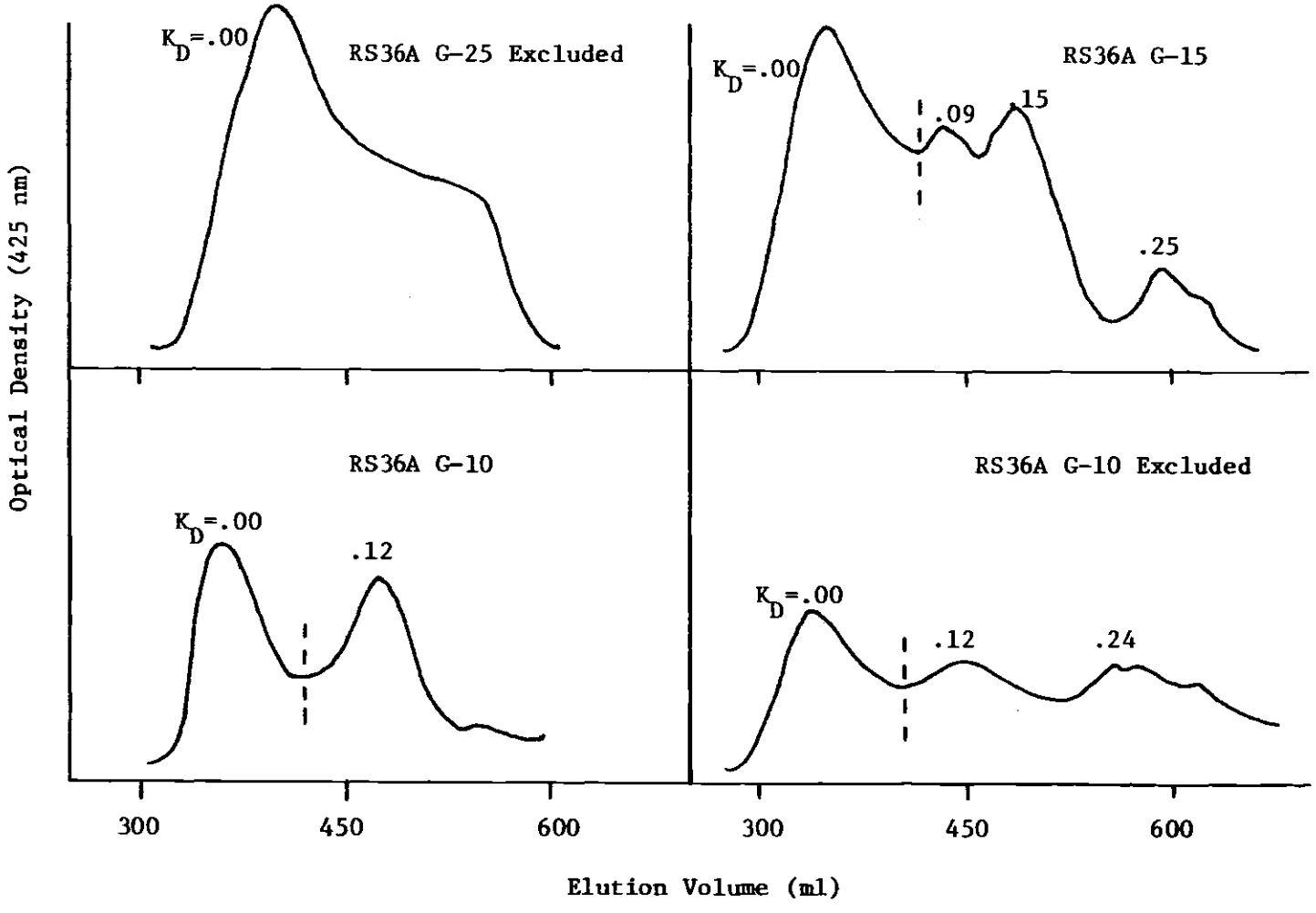

Figure 4 (Continued). Eluted Patterns of DOM. RS19 Fractions Eluted on 2.5 x 100 cm Column. RS36A Fractions Eluted on 4.5 x 50 cm Column.

material from G-25 Excluded was chromatographed and called G-25 Excluded II (Figure 3). The retarded portion of G-25 Excluded and the excluded portion of G-25 Excluded II (represented as dotted areas in Figure 4) were mixed and chromatographed as G-25X. The elution pattern of G-25X closely resembles the elution pattern of G-25. K_D values for the first four peaks of G-25 (K_D = .00, .21, .32, and .40, respectively) are very similar to K_D values for the first four peaks of G-25X (K_D = .02, .20, .31, and .44, respectively). However, in G-25, when the retarded material at K_D = .62 and .73 is removed, the elution pattern of material at K_D = .21, .32, and .40 changes drastically (see elution pattern for G-25 Excluded). This material is more greatly retarded. Since the characteristics of the gel remained the same throughout the fractionation on 'Sephadex' G-25, it is supposed that interactions between organic matter of varying heterogeneity occur. Probably differences in either steric configuration, oxygen-containing functional group content, or average molecular weight are influential in preventing fractionation solely on the basis of elution volume. Investigations by Reuter and Perdue (1972), and Rashid and King (1971) indicate that fractionation of humic substances on 'Sephadex' gels is related to the number of oxygen-containing functional groups in addition to the molecular weight.

Investigations of humic acid by Swift and Posner (1971) indicate that with adequate suppression of the interactions between oxygen-containing functional groups and 'Sephadex' gel a continuous fractionation,based only on differences of average molecular weight occurs. Elution of GFC fractions at pH 7 with distilled water does not sufficiently suppress interactions between functional groups and

gel. RS19,when fractionated on 'Sephadex' G-50 and eluted with a TRIS-HCl buffer solution at pH 9.0 (Figure 4),prepared according to Cameron et al. (1972), yielded an elution pattern similar to those obtained by Swift and Posner (1971). A continuous molecular weight distribution resulted.

Elemental Analysis

The elemental composition (C, H, N, and O) of humic substances from different environments is tabulated for comparison (Table 1). According to Schnitzer and Khan (1972), the percent distribution of carbon, hydrogen, nitrogen, and oxygen in humic acids varies between 50-60%, 4-6%, 1-3%, and 30-35%, respectively; for fulvic acid, the percent distribution is C 40-50%, H 3-6%, N less than 1-3%, and O 44-50%. Dissolved organic matter (DOM) in river water (Table 2) has the following composition: C 48-52%, H 3-4%, N 1%, and O 42-47% which closely resembles the composition of fulvic acid. The oxygen content of DOM fractions varies from 40% to 56%. Oxygen values in this range are characteristic of fulvic acid, and as discussed later, are proportional to the concentration of oxygen-containing functional groups. Although the organic matter was not analyzed for sulfur, it is presumed that based on numerous analyses by other investigators, the sulfur content would be less than one percent and would affect the calculated oxygen values only slightly.

Analyses of the fractions excluded on 'Sephadex' G-50 yielded elemental compositions similar to humic acid. Compared to the unfractionated material and their GFC fractions, the samples excluded on 'Sephadex' G-50 were high in carbon, low in oxygen, and insoluble in water after desalting and freeze-drying.

Table 1. Chemical Characteristics of Humic Substances from Different Environments

Environment and Type	Elemental Analysis (%)[3]					Oxygen-Containing Functional Group Analysis (meq/g)[3]					Reference
	C	H	N	O	Ash	TA[2]	Carboxyl	ØOH[2]	Carbonyl	Alcoholic OH	
Soil[1]: HA[2]	59.2	5.1	3.0	32.3		7.4	4.5	2.9	2.9	2.5	Riffaldi and Schnitzer (1972)
Humin	53.9	5.8	4.9	33.3		5.3	3.1	2.2	5.1		Riffaldi and Schnitzer (1972)
FA	45.3	5.8	2.7	45.4		8.5	6.6	1.9	3.7	5.4	Riffaldi and Schnitzer (1972)
FA	47.0	4.4	1.5	46.4		12.8	8.9	3.9	2.0	4.0	Schnitzer and Khan (1972)
HA synthesized by microbes	53.8	7.3	7.0	28.9							Riffaldi and Schnitzer (1972)
Lake Sediment: HA	50.4	5.9	8.1	35.6							Ishiwatari (1969)
Marine Sediment: HA	55.0	6.2	4.9	33.9		3.0	2.5	0.5	3.0	4.0	Rashid et al. (1972)
Bay Sediment: HA	54.8	5.9	4.0	33.3	17.2						Berryhill et al. (1972)
FA[2]	41.9	5.5	4.3	47.3	29.7						Berryhill et al. (1972)
River Sediment: HA	57.6	4.7	3.7	32.2	11.6						Berryhill et al. (1972)
FA	41.9	4.5	2.7	49.1	33.5						Berryhill et al. (1972)
Water: FA	48.5	4.2	1.1	46.2		12.4	8.9	3.5			Beck et al. (1973)
FA	46.2	5.9	2.6	45.3							Leenheer and Malcolm (1973)

[1]Average of several analyses.

[2]FA = fulvic acid, HA = humic acid, TA = total acidity, ØOH = phenolic hydroxyl.

[3]Expressed on a dry, ash-free basis.

Table 2. Elemental Analysis of DOM

Sample[1]	C	H	N	O[2]	Ash
RS19 Parent[3]	49.37	4.00	1.14	45.49	15.60
G-50 Excluded	52.17	4.07	1.20	42.55	3.58
G-25 Excluded	50.74	3.44	0.92	44.87	2.08
G-15 Excluded	50.38	3.57	0.91	45.19	2.39
G-15 Retarded A	39.95	4.04	0.78	55.26	1.15
G-15 Retarded B	43.06	3.94	1.04	51.96	1.89
G-15 Retarded C	53.79	4.44	1.17	40.60	4.57
RS36A Parent[3]	52.07	3.58	0.77	43.54	2.30
G-50 Excluded[3]	54.28	4.67	1.39	39.63	10.75
G-25 Excluded[3]	51.38	3.63	0.72	44.24	0.92
G-15 Excluded[3]	50.33	3.69	0.79	45.20	0.89
G-10 Excluded[3]	50.36	3.76	1.05	44.84	1.08
G-10 Retarded[3]	51.84	4.25	0.75	43.17	0.83
Raw[3]	49.35	3.69	0.82	46.15	7.65
Raw PPT[3]	49.65	5.64	1.91	42.82	39.13
RS36B Raw[3]	48.17	3.85	1.08	46.85	15.56

[1]Percent of C, H, N, and O expressed on an ash-free basis.

[2]Percent O calculated by difference.

[3]Data represent average of duplicate analyses.

The ash content of RS36A RAW was 50% lower than that of RS19 Parent; however, the material removed as precipitate (RS36A RAW PPT) had about twice the ash content of RS19. Removal of this material (RS36A RAW PPT) from the original material (RS36A RAW) probably accounts for the low ash content of RS36A Parent. RS36B RAW had an ash content comparable to the ash content of RS19 Parent. Infrared spectra and X-ray diffraction data of RS19 showed that most of the ash is present as SiO_2 and is unaffected by desalting with ion exchange resin. In other samples the ash probably consists of sodium, iron, and aluminum associated with DOM. It is interesting to note that the distribution of ash in the fraction of RS19 was fairly uniform (1.2%-4.6%) whereas in the fractions of RS36A most of the ash is concentrated in fraction G-50 Excluded. Results from infrared spectra indicate that the ash content of G-50 Excluded is mostly SiO_2.

Oxygen-Containing Functional Group Analysis

Samples RS19, RS36A, and their fractions were analyzed for total acidity and carboxyl group content (Table 3). Phenolic hydroxyl content was calculated by difference. A direct relationship between oxygen content (Table 2) and carboxyl group content was found for sample RS19 and its fractions. In most cases, total acidity and carboxyl group content increase as molecular weight decreases. However, the last fraction to elute on 'Sephadex' G-15, G-15 Retarded C, though exhibiting high total acidity (12.09 meq/g), showed a strikingly low carboxyl group content (5.37 meq/g), and a high phenolic hydroxyl content (6.72 meq/g), probably signifying the presence of polyphenolic material of low molecular weight such as tannins. The oxygen content of G-15 Retarded C was the lowest of all RS19

Table 3. Oxygen-Containing Functional Group Analysis (meq/g)[1]

Sample	Total Acidity	Carboxyl Groups	Phenolic Hydroxyl[2]
RS19 Parent	10.97	8.31	2.66
G-50 Excluded	8.90	4.47	4.43
G-25 Excluded	10.60	6.37	4.23
G-15 Excluded	10.77	6.97	3.73
G-15 Retarded A	12.92	9.53	3.39
G-15 Retarded B	11.94	9.19	2.75
G-15 Retarded C	12.09	5.37	6.72
RS36A Parent	10.35	6.17	4.18
G-50 Excluded	7.33	3.21	4.12
G-25 Excluded	10.64	6.09	4.55
G-15 Excluded	10.07	6.91	3.16
G-10 Excluded	11.96	7.74	4.22
G-10 Retarded	9.86	6.74	3.12
RS36A RAW	12.84	5.22	7.62

[1]Data expressed on an ash-free basis.

[2]Phenolic hydroxyl calculated by difference.

fractions (40.60%).

Total acidity and carboxyl group content of RS36A are similar to those of RS19, although the values for RS36A are slightly lower. The sum of the weights of the RS36A fractions (before removal of Na) represents a 100.5% yield of material after fractionation (Table 4). After desalting, the sum of the weights of the fractions totals to 82.3%. Percent recovery of material as calculated by the weight ratios of the fractions before and after desalting of RS36A was: G-50 Excluded 95.7%, G-25 Excluded 86.2%, G-15 Excluded 35.1%, G-10 Excluded 76.7%, and G-10 Retarded 70.2%. With the exception of G-15 Excluded, material of lower molecular weight appears to be preferentially adsorbed on the ion exchange resin. The weight distribution of the undesalted material (Table 4) is probably more representative of the true distribution of dissolved organic matter. The oxygen content of RS36A Parent and its fractions (with the exception of G-15 Excluded) is directly proportional to the total acidity.

Total acidity of RS36A RAW was higher than the total acidity of RS36A Parent, probably because of loss of low molecular weight material during desalting. The carboxyl group content of RS36A RAW was lower than for RS36A Parent. It would seem likely that exchangeable cations or chelated metals have blocked carboxyl reaction sites. Consequently, the phenolic hydroxyl content of RS36A RAW (7.62 meq/g) obtained by difference is inaccurate. Stevenson and Goh (1972) have reported evidence from infrared spectra for the nonspecificity of analytical methods for oxygen-containing functional groups, in particular carboxyl groups. Because of the wide variability of the acidic properties of COOH and OH groups

Table 4. Weight Distribution of DOM Fractions

Sample	Weight (g.)	% of Total
RS19 Parent	1.0000	
G-50 Excluded	0.1062	19.5
G-25 Excluded	0.0772	14.1
G-15 Excluded	0.0741	13.6
G-15 Retarded A	0.1004	18.4
G-15 Retarded B	0.1521	27.9
G-15 Retarded C	0.0358	6.6
	0.5458 (Total)	
RS36A[1] Parent	6.2900	
G-50 Excluded	0.2387	4.6
G-25 Excluded	2.8021	54.1
G-15 Excluded	0.3472	6.7
G-10 Excluded	0.7723	14.9
G-10 Retarded	1.0175	19.7
	5.1778 (Total)	
RS36A[2] G-50 Excluded + G-50 Retarded	6.8881	
G-50 Excluded	0.2494	3.6
G-25 Excluded	3.2524	47.0
G-15 Excluded	0.9896	14.3
G-10 Excluded	1.0063	14.5
G-10 Retarded	1.4255	20.6
	6.9232 (Total)	

[1]Desalted, weight determined after desalting and freeze-drying.

[2]Not desalted, weight determined after freeze-drying, but before desalting.

of humic substances, dissociation of these groups may overlap. Carboxyl groups strongly chelated do not readily dissociate. Other carboxyl groups may be more conveniently arranged to allow loss of acidic protons. Results of oxygen containing functional group analysis should therefore be interpreted with caution, particularly when obtained from organic matter high in ash content, e.g. RS36A RAW.

Infrared Spectroscopy

Infrared spectra of humic substances are characterized by broad overlapping absorption bands, in contrast to the well defined spectra of organic compounds found in living matter (Schnitzer and Khan, 1972). Absorption bands of humic acid in the 2900-2800 cm^{-1} region are stronger than those of fulvic acid indicating greater abundance of aliphatic groups (CH_2, and CH_3). In the 1725 cm^{-1} region fulvic acid has considerably stronger absorption bands indicating higher content of carboxyl groups. Infrared spectra of various humic substances can be classified into three spectral types (Stevenson and Goh, 1971). Characteristic absorption bands for each spectral type are as follows:

Type	Strong Absorption Bands (cm^{-1})	Weak or Absent Bands (cm^{-1})
I	1720, 1600	1640
II	1720	1650, 1600
III	1540, 1050	

Type I is characteristic of most humic acids, Type II is characteristic of low-molecular weight fulvic acid, and Type III exhibits bands which suggest the presence of proteins and carbohydrates. Results from this study indicate that

spectra of most DOM samples (Figure 5) are of Type II, whereas spectra of the GFC fractions (Figures 6 and 7) with the highest molecular weight (RS19 and RS36A G-50 Excluded) closely resemble humic acid of Type I. The major absorption bands are: 3400-3420 cm^{-1}: H-bonded OH groups including COOH; 2900 cm^{-1}: aliphatic C-H stretching; 1720-1725 cm^{-1}: C = O of COOH, C = O stretch of ketonic or aldehyde C = O; 1630-1625 cm^{-1}: COO^{-}, aromatic C = C, H bonded C = O of carbonyl; 1400 cm^{-1}: COO^{-}, aliphatic C-H, OH deformation, and C-O stretching of phenolic OH; 1200 cm^{-1}: C-O stretching and OH deformation of COOH groups; and 1050 cm^{-1}: C-O stretching of substances resembling polysaccharides and Si-O stretching of silicate present as an impurity.

Infrared spectra of RS19 and RS36A (Figure 5) indicate that dissolved organic matter is chemically related to fulvic acid. Similar absorption bands are found at 3400 cm^{-1}, 1725 cm^{-1}, 1625 cm^{-1}, 1400 cm^{-1}, and 1200 cm^{-1} reflecting similar carboxyl group content. Strong absorption in the 1050 cm^{-1} region of RS19 is believed to be the result of a silicate impurity (SiO_2). Sample RS36A RAW represents RS36A before purification and desalting. In the 1725 cm^{-1} region absorption of RS36A RAW is much weaker than absorption of RS36A, probably due to exchangeable and complexing metals on carboxyl groups. The spectrum of RS36B RAW shows only a shoulder at 1725 cm^{-1}, and the band at 1200 cm^{-1} is absent. Characterized by strong absorption at 1400-1395 cm^{-1} due to COO^{-} ions, the spectrum of RS36B RAW resembles the spectrum of the sodium salt of fulvic acid (Stevenson and Goh, 1971). RS36A RAW was collected during a period of swamp flushing, RS36B RAW on the other hand, was collected

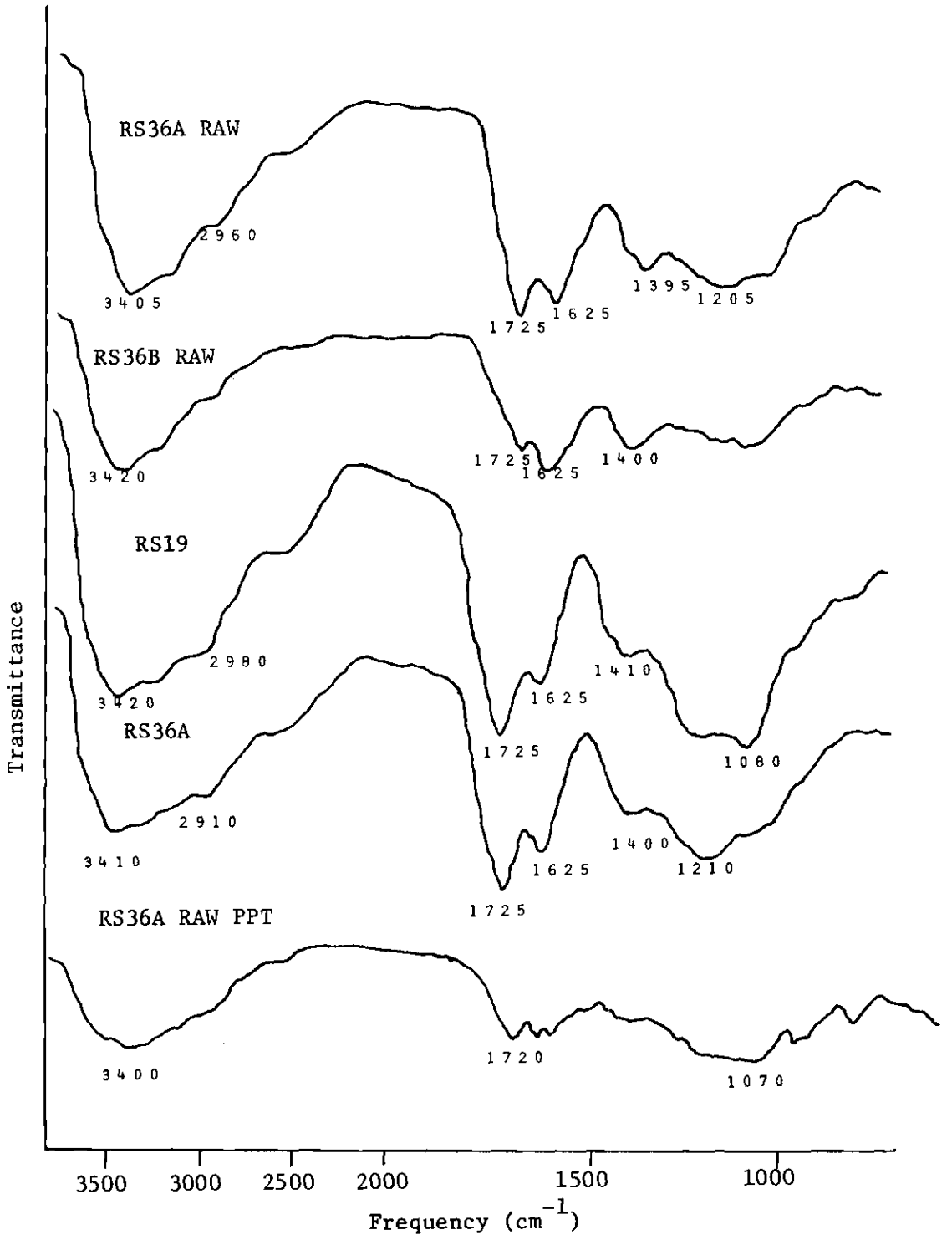

Figure 5. Infrared Spectra of DOM.

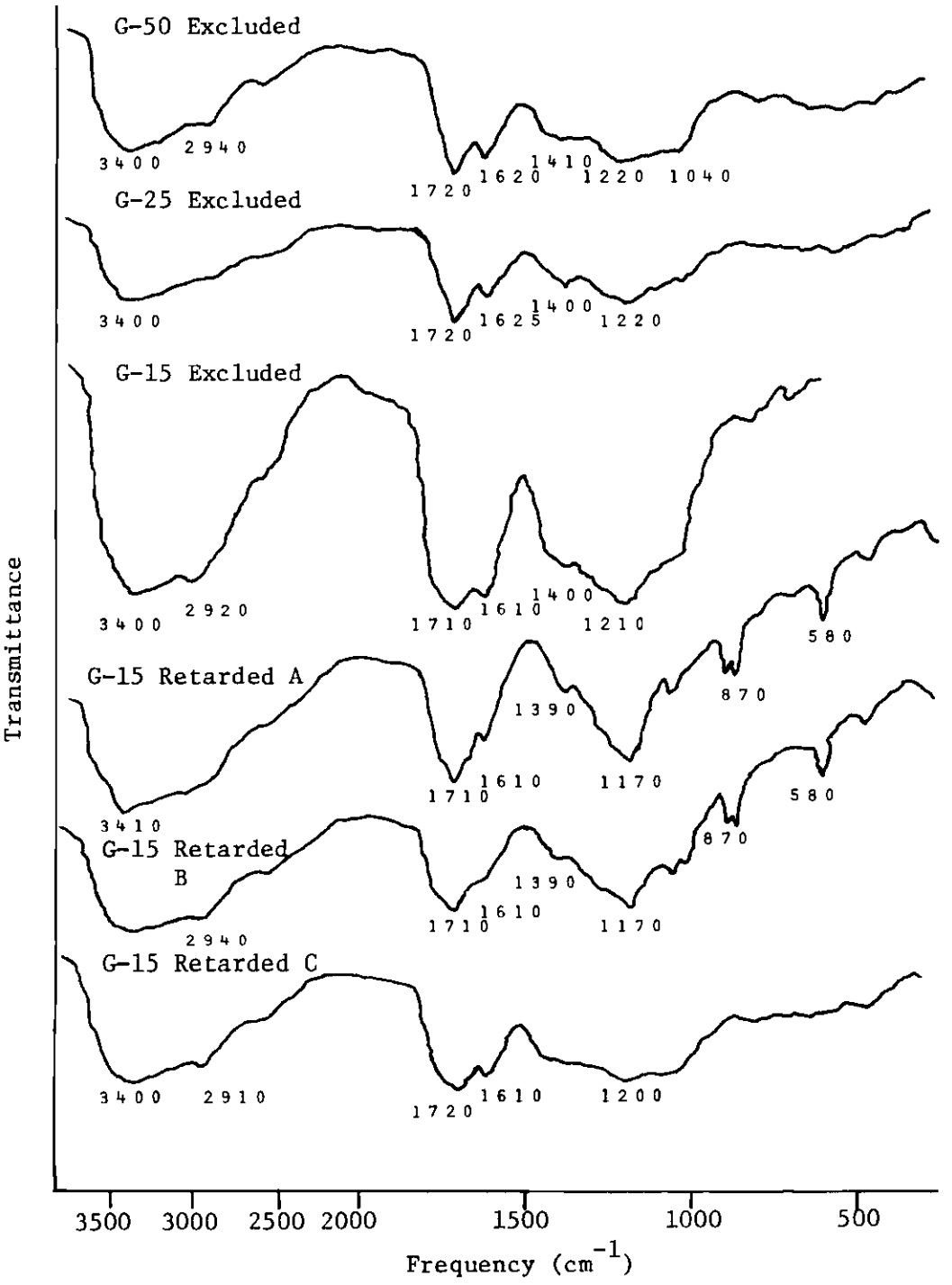

Figure 6. Infrared Spectra of RS19 Fractions.

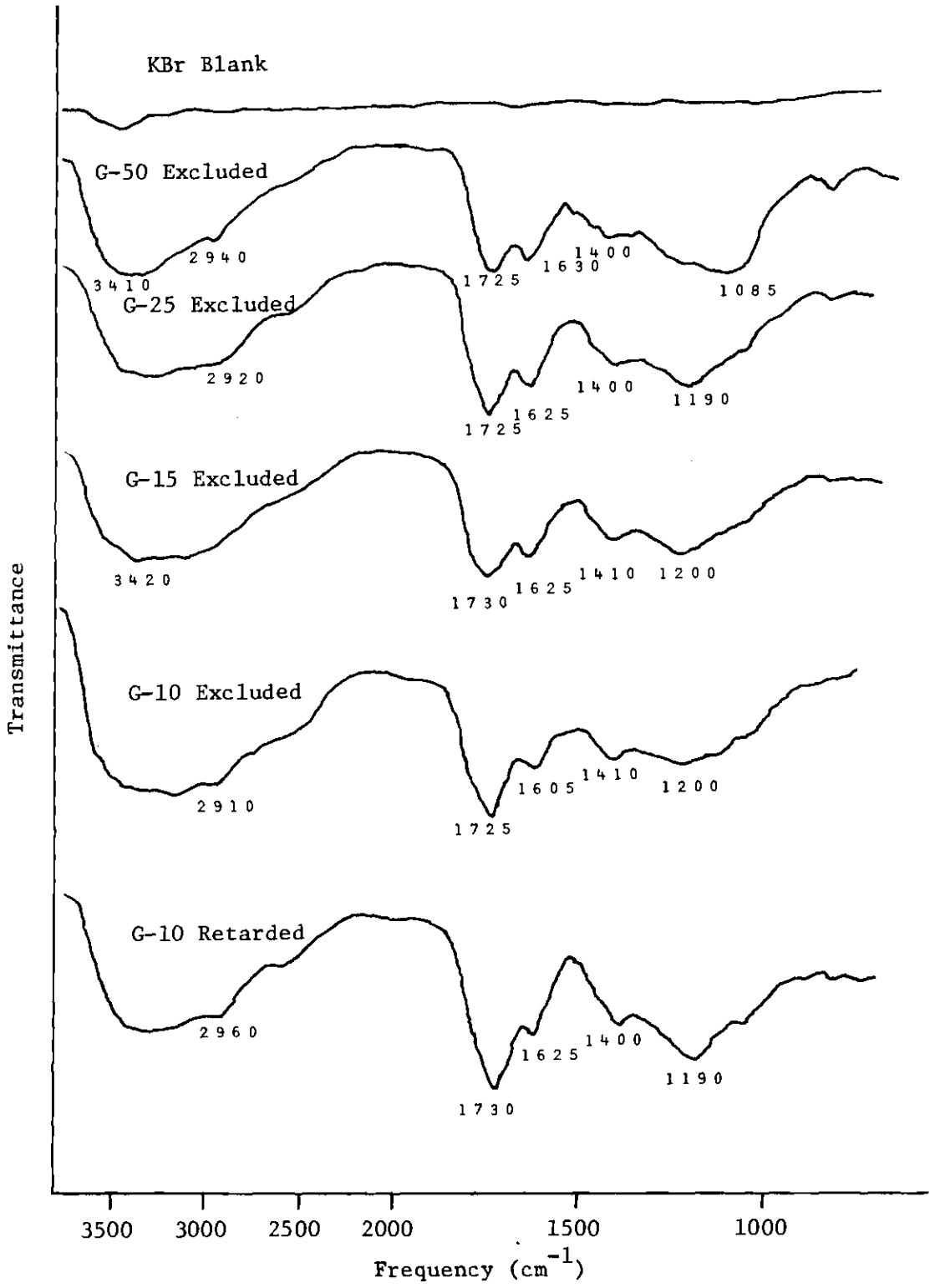

Figure 7. Infrared Spectra of RS36A Fractions.

during a stagnant period. The organic matter (RS36A RAW) with lower ash content (7.65%) was probably transported mostly by surface runoff. Containing a higher ash content (15.56%), RS36B RAW may have been leached from the soil primarily by subsurface drainage.

RS36A RAW PPT shows very broad absorption bands throughout its spectrum due to its high ash content (39.13%). The majority of absorption bands found in the spectrum of RS36A are present in the spectrum of RS36A RAW PPT, but are weaker, probably because of interactions between the organic matter and inorganic salts.

Infrared spectra of RS19 fractions (Figure 6) show the same broad absorption bands as those of RS 19 (before fractionation) due primarily to carboxyl and hydroxyl groups. Spectra of RS19 fractions, G-15 Retarded A and G-15 Retarded B exhibit similar bands in the 1000-500 cm^{-1} region attributable to aromatic substitution (Schnitzer and Khan, 1972). These spectra show strong absorption at 1725 cm^{-1} and 1200 cm^{-1} and reflect the high carboxyl group content of these two fractions (9.53 meq/g, and 9.19 meq/g, respectively).

Infrared spectra of RS36A fractions (Figure 7) are very similar to the spectrum of RS36A Parent. In general, fractions from G-50 Excluded to G-10 Retarded are characterized by increasing carboxyl group content (with the exception of G-10 Retarded), decreasing molecular weight, and increasing absorption at 1725 cm^{-1}. The above relations are consistent with findings by Stevenson and Goh (1971) and Schnitzer and Skinner (1968) on soil humic substances.

Number-Average Molecular Weight and Summary Formulae

The number-average molecular weights ($\overline{Mn}$) of DOM fractions ranged from 3095 to 528 (Table 6). The $\overline{Mn}$ of RS36A Parent was determined to be 1269. Since only a small amount of fraction G-50 Excluded was available, a $\overline{Mn}$ for this sample could not be determined accurately. The high $\overline{Mn}$ of G-50 Excluded (3095) supports results previously mentioned indicating that this fraction resembles humic acid.

In order to verify the reliability of $\overline{Mn}$ of dissolved organic matter, the $\overline{Mn}$ of a polycarboxylic acid of known molecular weight, benzenepentacarboxylic acid (BPCA) was determined, resulting in a $\overline{Mn}$ of 301 which compares favorably with the actual $\overline{Mn}$ of 298. The process used to calculate $\overline{Mn}$ from the data in Table 5 is described in detail by Hansen and Schnitzer (1969). ΔR and pH readings were corrected by the least squares method (Table 5).

$\overline{Mn}$, weight-average molecular weight ($\overline{Mw}$), and z-average molecular weight ($\overline{Mz}$) of the parent material were calculated according to Rafikov, et al. (1964) as follows:

$$\overline{Mn} = \Sigma\, MxVx = \frac{1}{\Sigma \frac{fx}{Mx}}\ ;\ \overline{Mw} = Mxfx;\ \text{and}\ \overline{Mz} = \frac{\Sigma Mx^2 fx}{\Sigma Mxfx}$$

where Mx = molecular weight of fraction x, Vx = number fraction, and fx = weight fraction. $\overline{Mn}$ calculated from GFC fractions is 1322 which compares favorably with $\overline{Mn}$ (1269) determined for RS36A Parent (Table 6). $\overline{Mw}$ and $\overline{Mz}$ calculate to be: 1333 and 2045, respectively. The ratio of $\overline{Mn}$: $\overline{Mw}$:$\overline{Mz}$ is 1.000:1.008:1.547.

Table 5. Data for the Determination of Number-Average Molecular Weight ($\overline{Mn}$)

a[1]	$\Delta R_{average}$	$\Delta R/a$	pH	y x 10^3	$\overline{Mn}_{(a)}$	$\overline{Mn}_{(a)corr.}$
A. Benzenepentacarboxylic Acid ($\overline{Mn}$ 301)						
8.000	2.706	.3383	1.766	2.142	178.2	288.2
10.000	3.331	.3331	1.699	2.000	181.1	283.9
12.000	3.956	.3297	1.643	1.896	182.9	280.0
15.000	4.893	.3263	1.577	1.766	184.8	274.3
17.000	5.518	.3246	1.541	1.693	185.8	271.1
20.000	6.455	.3228	1.491	1.614	186.8	267.4
B. RS36A Parent ($\overline{Mn}$ 1269)						
8.000	.914	.1143	2.104	.9838	527.6	1097
10.000	1.213	.1213	2.030	.9333	497.2	928
12.000	1.511	.1259	1.967	.8991	479.0	841
15.000	1.959	.1306	1.894	.8510	461.8	761
20.000	2.705	.1353	1.796	.7998	445.7	693
C. RS36A G-50 Excluded ($\overline{Mn}$ 3095)[2]						
8.000	.6473	.0809	2.288	.6440	745.5	1434
12.000	.7333	.0611	2.186	.5430	987.1	2127
15.000	.9488	.0632	2.110	.5175	954.3	1886
D. RS36A G-25 Excluded ($\overline{Mn}$ 2224)						
8.000	.6360	.0795	2.175	.8354	758.6	2071
10.000	.7940	.0794	2.125	.7499	759.6	1765
12.000	.9520	.0793	2.076	.6995	760.5	1625
15.000	1.189	.0793	2.002	.6636	760.5	1535
17.000	1.347	.0792	1.953	.6555	761.5	1521
20.000	1.584	.0792	1.878	.6622	761.5	1536
E. RS36A G-15 Excluded ($\overline{Mn}$ 1064)						
8.000	1.015	.1269	2.075	1.052	475.3	951
10.000	1.258	.1258	2.022	.951	479.4	881
12.000	1.502	.1252	1.970	.893	481.7	845
15.000	1.866	.1244	1.891	.857	484.8	829

Table 5 (Continued). Data for the Determination of Number-Average Molecular Weight ($\overline{Mn}$)

a[1]	$\Delta R_{average}$	$\Delta R/a$	pH	y x 10^3	$\overline{Mn}_{(a)}$	$\overline{Mn}_{(a)corr.}$
F. RS36A G-10 Excluded ($\overline{Mn}$ 661)						
8.000	1.381	.1726	1.972	1.333	349.4	654
10.000	1.696	.1696	1.922	1.197	355.6	619
12.000	2.010	.1675	1.873	1.116	360.1	602
15.000	2.481	.1654	1.798	1.061	364.6	595
17.000	2.795	.1644	1.749	1.048	366.8	596
20.000	3.267	.1634	1.674	1.059	369.1	606
G. RS36A G-10 Retarded ($\overline{Mn}$ 528)						
8.000	1.525	.1906	2.014	1.210	316.4	513
10.000	1.907	.1907	1.963	1.089	316.3	483
12.000	2.288	.1907	1.912	1.021	316.3	467
15.000	2.860	.1907	1.834	.977	316.3	458
17.000	3.242	.1907	1.783	.970	316.3	456
20.000	3.814	.1907	1.706	.984	316.3	459

[1] a = weight of sample/1000g solvent.

[2] $\overline{Mn}$ based on concentrations, 12.000 and 15.000 only.

Table 6. Analytical Characteristics of DOM and Fractions

Sample	Weight Fraction	$\overline{Mn}$	% of $\overline{Mn}$ as Functional Groups	Summary Chemical Formula[1]
RS36A G-50 Excluded	.0378	(3095)[2]	20.9	$C_{130.4}O_{44.8}H_{122.2}N_{3.0}(COOH)_{9.6}(OH)_{12.8}$
G-25 Excluded	.4455	2224	35.1	$C_{81.7}O_{24.3}H_{57.0}N_{1.1}(COOH)_{13.5}(OH)_{10.1}$
G-15 Excluded	.0552	1064	36.5	$C_{37.3}O_{12.0}H_{28.6}N_{0.6}(COOH)_{7.4}(OH)_{3.4}$
G-10 Excluded	.1228	661	43.6	$C_{22.6}O_{5.5}H_{17.0}N_{0.5}(COOH)_{5.1}(OH)_{2.8}$
G-10 Retarded	.1618	528	35.7	$C_{19.2}O_{5.4}H_{17.2}N_{0.3}(COOH)_{3.6}(OH)_{1.7}$
		1322[3]		
RS36A Parent		1269[4]	34.9	$C_{47.3}O_{13.5}H_{32.3}N_{0.7}(COOH)_{7.8}(OH)_{5.3}$
Fulvic Acid[5]		951	46.5	$C_{30.6}O_{6.8}H_{31.2}N_{0.5}(COOH)_{8.7}(OH)_{3.1}$

[1] Number of OH groups calculated from phenolic hydroxyl content, not representative of total hydroxyl content.

[2] Based on limited data.

[3] $\overline{Mn}$ of Parent calculated from fractions.

[4] $\overline{Mn}$ of Parent calculated from vapor pressure osmometry.

[5] Data for soil fulvic acid from Bh horizon from Schnitzer and Skinner (1968) and Hansen and Schnitzer (1969).

For a monodisperse polymer, $\overline{Mn} = \overline{Mw} = \overline{Mz}$, but for a polydisperse (heterogeneous) system of polymers, $\overline{Mn} < \overline{Mw} < \overline{Mz}$. The ratio $\overline{Mw}/\overline{Mn}$ of RS36A is close to unity, indicating that the parent material is of low polydispersity.

For GFC fractions of soil fulvic acid, Hansen and Schnitzer (1969) obtained $\overline{Mn}$'s ranging from 2110 to 275. Most of the soil fulvic acid (59%) was found in the excluded material on 'Sephadex' G-15, whereas the major fraction of dissolved organic matter (47%) was excluded on 'Sephadex' G-25. Soil fulvic acid appears to be a more polydisperse system $\left(\overline{Mn}/\overline{Mn} = 1.19\right)$ when compared to dissolved organic matter $\left(\overline{Mw}/\overline{Mn} = 1.008\right)$. The difference in distribution and polydispersity is probably related to the extraction method. Fulvic acid represents organic matter extracted from soil by alkali, e.g. NaOH, whereas river water organic matter can be regarded as humic substances naturally leached from soil and swampy areas by percolating waters.

Although numerous studies have been concerned with the determination and distribution of molecular weights of humic substances from various environments, few investigations have yielded comparable results. The difficulties associated with molecular weight determinations as cited by Orlov, et al. (1971) include the difference in molecular size, variability of similarly sized molecules in composition, the dynamic state of change in size and composition (humification), and interactions between like or different molecules by hydrogen bonding and cation bridging. In general, molecular weights of humic substances cover a wide range varying from several hundred to several hundred thousand. The high molecular weight fraction of humic substances, humic acid, varies from 1,000 to

to 200,000 (Schnitzer and Khan, 1972). The molecular weight distribution of humic substances in natural waters ranges from several hundred to several thousand (Brogden, 1971, Ghassemi and Christman, 1968, Gjessing and Lee, 1967, and Shapiro, 1957). Most of these studies used gel filtration to determine average or apparent molecular weight of dissolved organic matter. 'Sephadex' gels were calibrated by substances of known molecular weights such as proteins, peptides, and dextrans. It appears that gels calibrated in this fashion yield average molecular weights 2 to 10 times higher than those obtained by vapor pressure osmometry (Schnitzer and Skinner, 1968).

Summary formulae based on elemental composition, oxygen-containing functional group content, and $\overline{Mn}$ were calculated for RS36A and its fractions (Table 6). The number of hydroxyl groups was calculated from the phenolic hydroxyl content and is not representative of the total hydroxyl content. Other oxygen-containing functional groups such as alcoholic hydroxyl, methoxyl, and carbonyl groups were not determined for RS36A and though not included in the summary formulae are probably present in small amounts. The nitrogen content in the summary formulae is low and consists mostly of amino acid nitrogen (Beck et al., 1973). Oxygen-containing functional groups comprised from 20.9 percent to 43.6 percent of the $\overline{Mn}$.

CHAPTER IV

CONCLUSIONS

Based on chemical and physical characteristics, river water organic matter consists of humic substances, the bulk of which resembles fulvic acid. In areas of high rainfall and low relief such as the Satilla River Basin, large quantities of humic substances are produced from the degradation of abundant vegetative litter (Beck et al., 1973). Of the humic substances produced, only the more soluble, lower molecular weight material is carried into the river. Limited by its solubility in water, river water organic matter is of relatively low polydispersity when compared to other humic substances. As river water organic matter is transported downstream, the higher molecular weight portion (low in total acidity) resembling humic acid is removed, probably by flocculation. The dissolved organic matter transported to the estuary would be characterized by low molecular weight and high exchange capacity.

The results of this study have broad implications regarding environmental problems such as the toxic metal pollution of rivers and estuaries. Heavy industries and dense population centers have increasingly discarded waste products into the rivers. Heavy metals found as part of these waste products have the ability to form stable, water soluble organo-metallic complexes with humic substances (Schnitzer, 1971). Precipitation of heavy metals as insoluble salts is prevented by the presence of soluble humic substances (Rashid and Leonard, 1973).

In the Satilla River and other rivers, where dissolved organic matter resembling humic substances is abundant, its presence may play an important role in the transportation of heavy metals. Organic matter and metal-organic complexes are introduced to the estuary as either dissolved or colloidal material. In laboratory studies, the addition of an electrolyte such as NaCl to humic colloids causes coagulation (Ong and Bisque, 1968). Thus it is expected that when soluble organic matter and associated metals transported by river water encounter increased salinity in the estuary, flocculation occurs resulting in rapid deposition and accumulation of metal in salt marsh sediments.

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